

DNA FIBER PREPARATION TECHNIQUE ON A CHIP FOR CLINICAL DIAGNOSIS

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ABSTRACT

In this study, we propose a simple observation method of the shape and molecular orientation of the chromosomes extracted from the cell for a sample-to-analysis system in clinical diagnosis. The proposed technique is composed of total preparation technique such as cell immobilization, chromosomes extraction, stretching, suspension and analysis using a disposable microchip controlled by centrifugal force only. It is experimentally confirmed that the chip having two kinds of microstructures arranged concentrically on a chip is possible to immobilize cells and stretch chromosomes extracted from the immobilized cells.

KEYWORDS

Gene Analysis Chip, DNA fiber, Centrifugal force, Chromosome, Clinical Diagnosis, Photolithography.

INTRODUCTION

Several visualization methods for intact chromosomes without fragmentation were proposed to stretch the chromosomes extracted from living cells for high resolution observation with the fluorescent microscopy [1-4]. The conventional methods can precisely manipulate few chromosomes with complex operation, but are not suitable for observing the specific sequences in large number of chromosomes for clinical diagnosis. So, we have proposed the rotating microchip that has micromesh structure concentrically, examined the liquid inside suspending method, quantitative evaluated stretching shape of chromosomes and dyeing efficiency and performed validity verification using HeLa cells [1]. In this study, we propose a fully-integrated device performs to stretching of chromosomes from cell immobilization by rotating the device for a sample-to-analysis system in clinical diagnosis. Furthermore, we simultaneously fabricated the device composed of opened cone-structures trapping cells and micro-bridges suspending DNA fibers by the rotated/inclined exposure method with single step, evaluated efficiency of the cell immobilization, performed stretching chromosomes from immobilized cells.

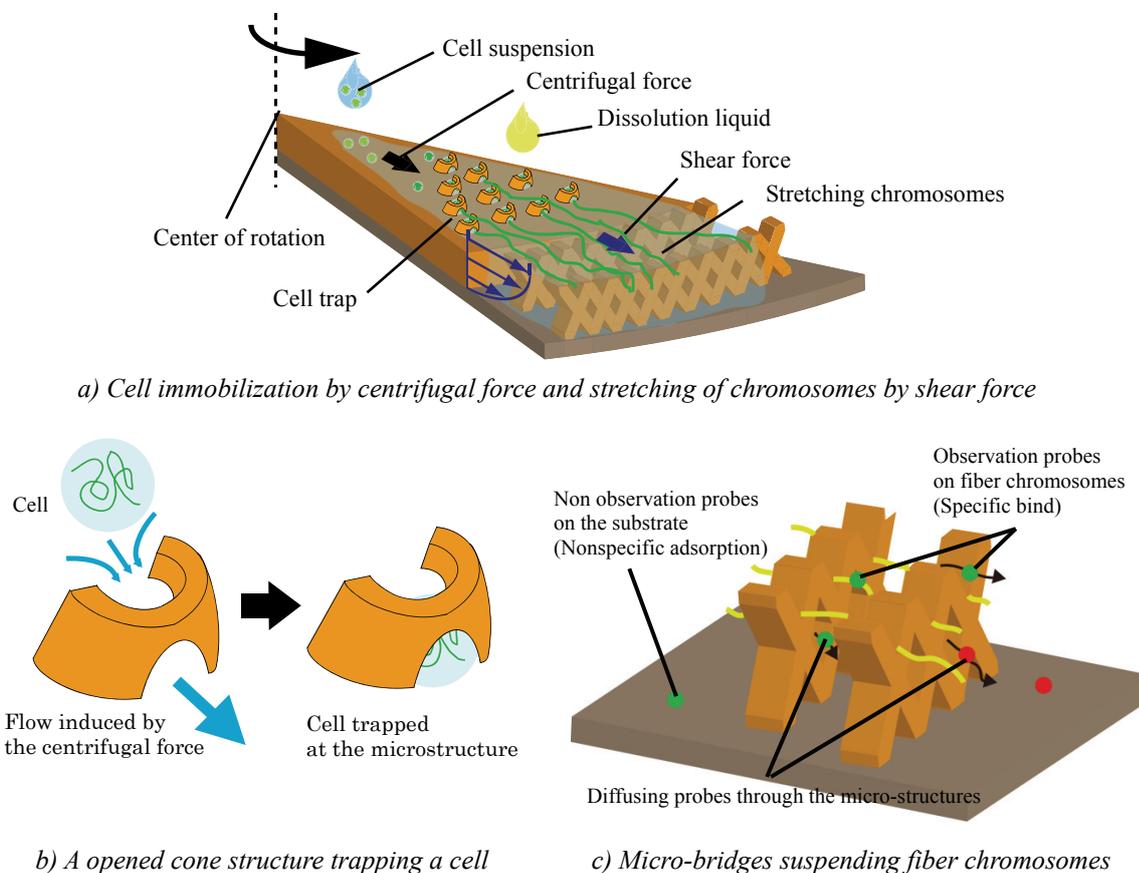


Figure 1. Schematic of DNA fiber preparation technique on a chip for a sample-to-analysis system in clinical diagnosis

PRINCIPLE OF CHROMOSOME STRETCHING

Figure 1 shows schematics of DNA fiber preparation technique on a disk-like chip for a sample-to-analysis system in clinical diagnosis. The proposed device applied to the centrifugal force as a principle for all the operations, can immobilize a number of cells at once, has the feature that can stretch the chromosomes from immobilized cells, simultaneously.

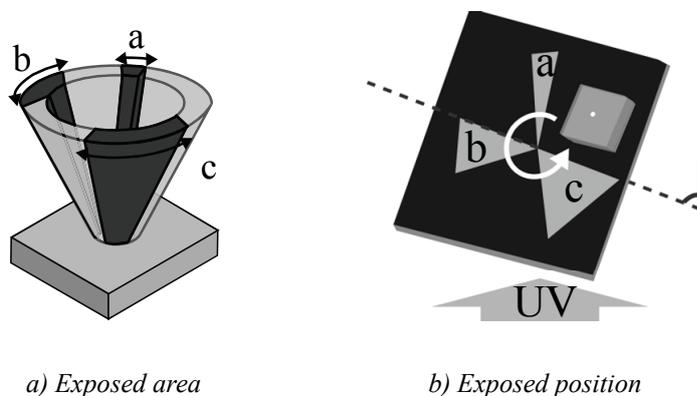


Figure 2. Principle of rotated/inclined exposure method with a fixed mask

FABRICATION PROCESS

The structures of the proposed device consists resin by the single process of the rotated/inclined exposure method as shown in Fig.2. The proposed process involves directly spin-coating a thick negative photoresist SU-8 on a patterned mask and rotational exposing it from the backside through a fixed mask. During the exposure process, the photoresist with the mask is continuously rotating under constant incident angle. The fixed mask over the rotated mask limits the exposure area on the rotated mask. A large variety of microstructures are fabricated by the combination of the mask patterns of the rotated and fixed masks. Figure 3 shows SEM images of the fabricated device, external diameter is 14mm, there are the cell traps and the mesh structures concentrically.

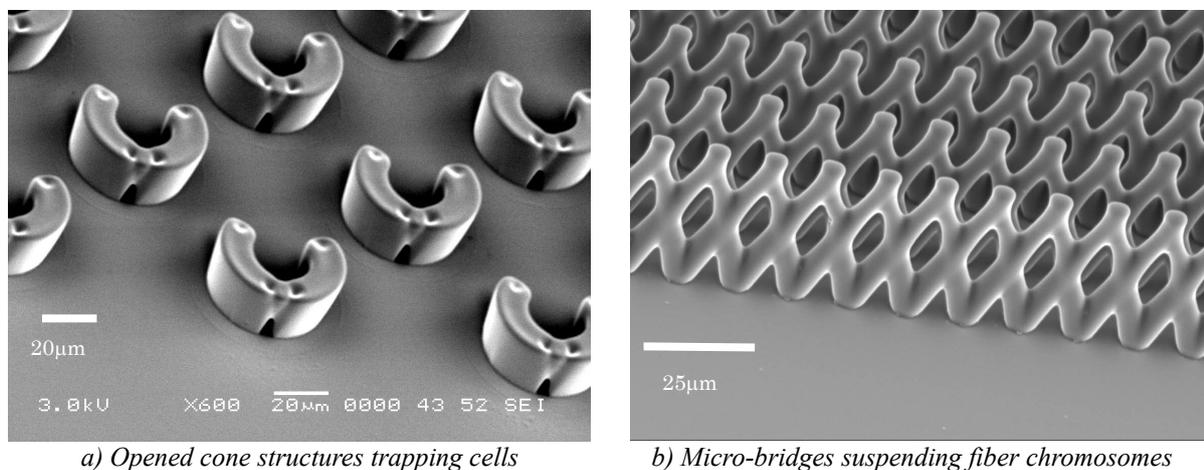


Figure 3. SEM images of the fabricated device

RESULTS AND DISCUSSION

For the experiment of the cell immobilization, the fabricated device could immobilize cells up to 600 cells by the centrifugal within 10 seconds. Also, figures 4 and 5 show a fluorescent image of stretching chromosomes extracted from the cell immobilization, and the chromosome stretching efficiency from the trapped cells, respectively. By controlling the spin speed and attaching a cover, the proposed device achieves 30% higher chromosome stretching efficiency from the trapped cells than the previous device.

To confirm the validity of the microchip, we compared concentric microstructures; flat, wall, and mesh/trap structures on the rotating microchips. The proposed micro-mesh structures with cell trap array have higher efficiency than the conventional methods, and can achieve high-throughput and steady detection of the specific sequences in large number of the chromosomes for clinical diagnosis with the simple technique.

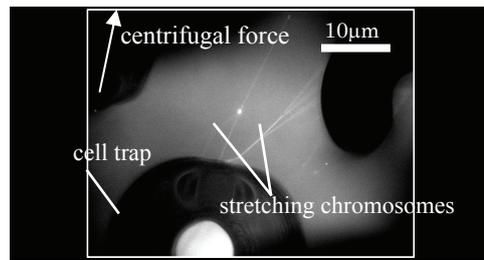


Figure 4. Fluorescent image of stretching chromosomes extracted from trapped cell

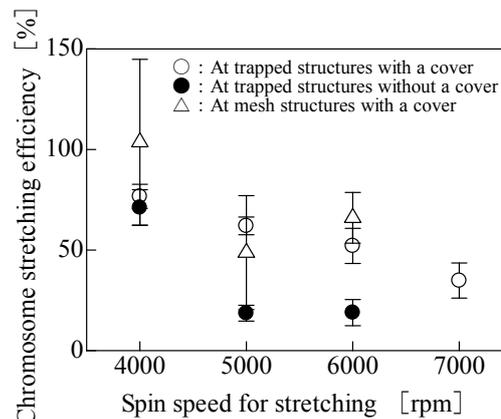


Figure 5. Chromosome stretching efficiency from the trapped cells

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ACKNOWLEDGEMENTS

This work was partly supported by the 2008 Industrial Technology Research Grant Program from New Energy and Industrial Technology Development Organization of Japan, Grant-in-Aid for Scientific Research(B) (23360114), and Specific measure promotion cost of Kagawa University - Special promotion research (Border cooperation between departments).

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